MC1R Germline Variants Confer Risk for *BRAF*-Mutant Melanoma

Maria Teresa Landi,[1]*† Jürgen Bauer,[2,3]* Ruth M. Pfeiffer,[1] David E. Elder,[4] Benjamin Hulley,[1] Paola Minghetti,[5] Donato Calista,[5] Peter A. Kanetsky,[7] Daniel Pinkel,[6] Boris C. Bastian[2]

Germline variants in *MC1R*, the gene encoding the melanocortin-1 receptor, and sun exposure increase risk for melanoma in Caucasians. The majority of melanomas that occur on skin with little evidence of chronic sun-induced damage (non-CSD melanoma) have mutations in the *BRAF* oncogene, whereas in melanomas on skin with marked CSD (CSD melanoma) these mutations are less frequent. In two independent Caucasian populations, we show that *MC1R* variants are strongly associated with *BRAF* mutations in non-CSD melanomas. In this tumor subtype, the risk for melanoma associated with *MC1R* is due to an increase in risk of developing melanomas with *BRAF* mutations.

Epidemiologic (*1*, *2*) and molecular (*3*, *4*) studies suggest that different types of human melanoma can be distinguished on sun-exposed skin. Tumors on skin with few or no histopathologic signs of CSD, as evidenced by the relative absence of solar elastosis in the surrounding skin, occur in younger individuals and have frequent mutations in the BRAF oncogene (non-CSD melanoma). BRAF encodes a serine/threonine kinase involved in the transduction of mitogenic signals from the cell membrane to the nucleus. By contrast, melanomas on skin with signs of CSD affect older individuals, have different patterns of chromosomal aberrations, and have a lower frequency of BRAF mutations (CSD melanoma) (*4*). Because melanomas on anatomic sites exposed to ultraviolet radiation (UVR) predominantly affect Caucasians, and non-CSD melanomas occur at relatively low UVR doses, we hypothesized that the high frequency of BRAF mutations in this melanoma type is due to a susceptibility factor(s) that occurs at higher frequencies in Caucasian populations (*4*).

A promising candidate susceptibility factor is the melanocortin-1 receptor (MC1R), a G-protein coupled receptor on melanocytes that responds to alpha-melanocyte stimulating hormone (α-MSH) secreted in response to UVR (*5*). The MC1R gene is highly polymorphic in Caucasians (*6*). Its sequence variants can result in partial (r) or complete (R) loss of the receptor's signaling ability, although the degree of functional loss of many MC1R variants is not accurately known. The variants contribute to distinct phenotypic traits such as fair skin, freckling, and red hair (*7*, *8*). Furthermore, MC1R variation has been shown to be a melanoma risk factor (*9*), even beyond its effect on pigmentation (*10*–*12*).

To determine whether there is an association between MC1R variants and BRAF-mutant melanoma, we sequenced the entire coding region of MC1R in germline DNA and the exon 15 of BRAF (where a mutation hot spot is located) in primary cutaneous melanomas from 85 patients from a case-control study conducted in Italy from 1994 to 1999 (*13*, *14*). We performed a similar analysis on an independent set of 112 invasive primary cutaneous melanomas examined at the Department of Dermatology at the University of California, San Francisco, in 2004 and 2005. The MC1R variants identified in the two populations are listed in table S1. The degree of solar elastosis in the skin adjacent to each tumor was assessed independently by two pathologists (*15*) using a multipoint scale from 0 to 3+ (fig. S1). There was good concordance between the two pathologists' scores (weighted kappa = 0.58 and 0.71 for the Italian and U.S. populations, respectively). For statistical analysis, melanomas were classified as non-CSD if they showed only minor signs of solar elastosis (CSD level 0 to 2–) (fig. S1) and as CSD if they had more pronounced solar elastosis (CSD levels 2 to 3+) (fig. S1). As expected, subjects with non-CSD melanomas were younger than those with CSD melanomas, and their tumors arose more frequently on intermittently sun-exposed anatomic sites (e.g., trunk) than on continuously exposed sites (e.g., face) (table S2).

BRAF mutations were more frequent in non-CSD melanoma cases with germline MC1R variants than in those with two wild-type MC1R alleles. When we categorized patients into two groups—homozygous MC1R wild-type versus all others—we found that BRAF mutations were 6 to 13 times as frequent in those with at least one MC1R variant allele compared to those with no MC1R variants (Table 1, upper half). Using a finer MC1R categorization with three groups (zero, one, or two variant alleles), the odds ratio for BRAF mutations in the non-CSD melanomas increased progressively (P = 0.001 and 0.02 for

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, MD 20892, USA. [2]Departments of Dermatology and Pathology and Comprehensive Cancer Center, University of California, San Francisco, CA 94143, USA. [3]Department of Dermatology, Eberhard Karls University, 72076 Tübingen, Germany. [4]Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA. [5]Department of Dermatology, M. Bufalini Hospital, Cesena, 47023, Italy. [6]Department of Laboratory Medicine, University of California, San Francisco, CA 94143, USA. [7]Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: landim@mail.nih.gov

Table 1. Association between inherited variants of *MC1R* and tumor-specific *BRAF* mutations in non-CSD melanomas. WT, wild type; R, *MC1R* variants with complete loss of function; r, *MC1R* variants with partial loss of function.

MC1R	Italy				United States			
	BRAF WT (row %)	*BRAF* mutant (row %)	Odds ratios (95% CI)*	*P*	*BRAF* WT (row %)	*BRAF* mutant (row %)	Odds ratios (95% CI)*	*P*
WT/WT	7 (70.0)	3 (30.0)	Reference		6 (66.7)	3 (33.3)	Reference	
Any variant	9 (19.6)	37 (80.4)	13.2 (2.1–81.4)	0.006	18 (36.7)	31 (63.3)	6.0 (1.2–30.6)	0.03
WT/WT	7 (70.0)	3 (30.0)	Reference		6 (66.7)	3 (33.3)	Reference	
r/WT or R/WT	8 (23.5)	26 (76.5)	10.6 (1.7–67.5)	0.01	15 (44.1)	19 (55.9)	4.1 (0.7–23.0)	0.11
r/r or R/r or R/R	1 (8.3)	11 (91.7)	38.6 (2.5–590.8)	0.009	3 (20.0)	12 (80.0)	10.6 (1.5–74.6)	0.02
Total	16 (28.6)	40 (71.4)		*P* trend = 0.001	24 (41.4)	34 (58.6)		*P* trend = 0.02

*Logistic regression models adjusted by age (quartiles).

Table 2. Melanoma risk in the Italian case-control study by inherited variants of *MC1R* and tumor-specific *BRAF* mutations in non-CSD melanomas. WT, wild type; R, *MC1R* variants with complete loss of function; r, *MC1R* variants with partial loss of function.

MC1R	Controls (No.)	Melanoma cases* (No.)			Odds ratios for melanoma risk (95% CI)†					
		All cases	*BRAF* WT	*BRAF* mutant	All cases	*P*, All cases	*BRAF* WT	*P*, *BRAF* WT	*BRAF* mutant	*P*, *BRAF* mutant
WT/WT	71	10	7	3	Reference		Reference		Reference	
Any variant	100	46	9	37	3.3 (1.5–6.9)	0.002	0.9 (0.3–2.5)	0.79	8.8 (2.6–29.8)	0.0005
WT/WT	71	10	7	3	Reference		Reference		Reference	
r/WT or R/WT	85	34	8	26	2.8 (1.3–6.1)	0.008	1.5 (0.2–13.3)	0.7	7.2 (2.1–24.9)	0.002
r/r or R/r or R/R	15	12	1	11	5.7 (2.1–15.6)	0.001	1.3 (0.2–11.8)	0.8	17.0 (4.2–68.6)	0.0001
Total	171	56	16	40		*P* trend = 0.0003		*P* trend = 0.88		*P* trend < 0.0001

*Only CSD negative cases are included in the analyses. †Logistic regression models adjusted by age (quartiles, in control subjects).

trend in the Italian and U.S. populations, respectively) (Table 1, lower half, and table S3). In an analysis stratified by median age, the association between MC1R and melanoma risk by BRAF mutation status was stronger in the younger subjects (table S4). However, formal tests for interaction between age and MC1R were not significant ($P = 0.22$ and $P = 0.13$ in the Italian and U.S. populations, respectively). MC1R variation had no effect on the frequency of BRAF mutations in melanomas with CSD, although the small number of CSD-positive subjects precluded a formal statistical analysis in the Italian group (table S5).

Comparison of the non-CSD Italian cases with 171 healthy Italian controls showed that the overall melanoma risk was higher by a factor of 3.3 [95% confidence interval (CI) 1.5 to 6.9] in individuals with any MC1R variant allele compared to individuals with no variant alleles and that the risk increased with the number of variant MC1R alleles (Table 2). By stratifying the tumors on the basis of the presence or absence of BRAF mutations, it became evident that the risk was confined to the melanomas with BRAF mutations. The odds ratio increased from 7.2 (95% CI = 2.1 to 24.9) for individuals with one MC1R variant allele to 17.0 (95% CI 4.2 to 68.6) for those with multiple variant alleles when compared with individuals with no MC1R variants ($P < 0.0001$ for trend across categories) (Table 2 and table S6). These results remain significant when using a Bonferroni correction for multiple testing. BRAF mutations were not associated with phenotypic characteristics that are usually associated with sun sensitivity, such as hair color, eye color, spectrophotometrically assessed skin color (*15*), and tanning ability (see table S7 for a comprehensive list).

The relation between BRAF mutations in melanoma and sun exposure is complex and intriguing. On the one hand, sun exposure appears necessary for the development of BRAF mutations because melanomas on mucosa-lined body cavities, the soles, the palms, and subungual sites have low mutation frequencies (11 to 23%) compared to the ~60% mutation frequency in non-CSD melanoma (*4*). On the other hand, melanomas developing in older subjects, after accumulated sun exposure sufficient to produce CSD in the surrounding skin, also exhibit lower BRAF mutation frequencies, arguing against a simple link between UVR exposure and BRAF mutation. Moreover most BRAF mutations do not show the standard C > T signature of direct UVR induction. This paradoxical relationship motivated our hypothesis that there is an inherited susceptibility factor(s) that predisposes individuals to develop BRAF-mutant melanoma under limited sun exposure or earlier in life and that UVR may act indirectly to promote these mutations.

Our results show that variant alleles of MC1R are at least one component of this hypothesized susceptibility. BRAF mutations are a characteristic feature of more than 80% of the non-CSD melanomas in individuals with two variant MC1R alleles but only in ~30% of individuals with wild-type MC1R (Table 1). The mechanism mediating this susceptibility is currently unknown; however, previous studies suggest that it may in part be independent of pigmentation (*10*–*12*). One possibility is increased generation of reactive oxygen species in carriers of MC1R variants (*16*), which could be independent of pigmentation (*17*) and directly induce the A > T transversion characteristic of the common BRAF V600E mutation in exon 15.

Epidemiological studies often identify associations between cancer risk and environmental exposures, but tumors developing in response to comparable environmental exposure frequently show a variety of somatic changes. Such differences may be due to the stochastic nature of mutation coupled with selection during tumor development. Alternatively, as we show here, the difference may be due to specific inherited genetic variants. Our discovery of the MC1R-BRAF relationship was dependent on careful classification of melanomas into CSD and non-CSD subtypes. We expect that similar subtyping of other cancers will reveal important associations of environmental exposures with germline variants and somatic genetic alterations.

References and Notes

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Supporting Online Material

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Materials and Methods
Tables S1 to S8
Fig. S1

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